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Comparative Study of Phytochemicals and Antioxidant Activity of Selected Plants of Family Apocynaceae

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Abstract : *Methanolic extract of Vinca rosea leaf demonstrated high total antioxidant capacity as compared to Thevitia peruviana. However, total antioxidant capacity of flower of Thevitia peruviana is more than that of Vinca rosea. Thevitia peruviana leaf showed highest absorbance i.e high reducing power as compared to Vinca rosea leaf, whereas Vinca rosea flower showed high reducing power than Thevitia peruviana flower.The antioxidant property is concentration dependent .The result obtained in this study indicated that antioxidant property of Thevitia peruviana was nearly equal to Vinca rosea which is a well known medicinal plant. Hence this member of the family can also be considered for antioxidant source.*

Key Words : *Antioxidants, tannins, hydrogen peroxide, phytochemical.*

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Introduction :

Plants have been a rich source of important therapeutic agents and form the basis of herbal systems of medicine, like ayurveda, resulting in the revival of ancient traditions of medicine. The present study was carried out to investigate the anti- oxidant potential of the leaves and flowers of plants of family Apocynaceae using *in vitro* model systems like Hydrogen peroxide scavenging activity, Total anti oxidant capacity and Reducing Power Assay.

Production of free radicals in the biological system results the imbalance in the level of pro-oxidant and antioxidant known as oxidative stress. In living organisms oxygen in unstable form is the most common free radical. This is called Reactive Oxygen Species (ROS) and is generated during various metabolic activities. These ROS are able to oxidize cellular bio-molecules like nucleic acids, proteins, lipids and carbohydrates (Borah *et al*., 2011). Antioxidants are radical scavengers which give protection to human body against free radicals by inhibiting the oxidizing chain reactions. When these substances are present at low concentration in body they markedly delay or prevent the oxidation of an oxidizable substrate. These

antioxidants always play important roles in delaying the development of chronic diseases such as, cardiovascular diseases (CVD), cancer, atherosclerosis, inflammatory bowel syndrome and Alzheimer's diseases (Rasool *et al*., 2011)

Plants are one of the important sources of medicines. Plants have been known since ancient time for their medicinal uses. Plants develop several antioxidants that aid in antioxidant defense system, protecting plants against damage caused by active O_2 formed due to exposure to ultraviolet radiation. Our daily diet contains vegetables, fruits, tea, juices etc which possess compounds reach in anti oxidative properties (Kumar *et. al*., 2008). Plant derived natural products such as, flavanoids, terpenoids, and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activities.

In the present study, an attempt has been made to evaluate the antioxidant and radical scavenging activities of some plants of the family Apocynaceae by different *in vitro* models.

Materials and Methods :

Collection of plant material: Plants of *Vinca rosea* and *Thevetia peruviana* were collected from localities of Patna and were maintained in the herbarium sheets for reference with voucher specimen no. as PWC/BOT/04 and PWC/BOT/05 for *Vinca rosea* and *Thevetia peruviana,* respectively.

Preparation of extract: Leaves and flowers of selected plants were dried under sun and then grinded in a grinder to make fine powder. Then 1g powder of each of *Vinca* leaves and flowers as well as *Thevitia* leaves and flowers were dissolved in 50 ml distilled water and 50 ml methanol separately and mixed with continuous stirring and kept overnight. Then the filtrates were centrifuged at 5000 rpm for 10 min. The supernatants were then kept in hot air oven for 24 h at 45^0 C for solvent evaporation. At last each extract were weighed and mixed with distilled water and methanol and stored in air tight bottles at 4^0C for further use.

Phytochemical screening of plant extracts: The methanolic and aqueous extracts of the plants were subjected to preliminary phytochemical testing for the detection of major chemical groups i.e. Tannins, Flavanoid, Alkaloid Terpenoid, Steroid, Saponin and Catechins, Cardiac glycosides Phytochemical screening of the extracts was performed using the standard procedures (Harborne, 1973).

Hydrogen peroxide scavenging activity: Ability of the extracts to scavenge hydrogen peroxide was determined One ml of the extract was rapidly mixed with 2 ml of 10 mM phosphate buffered (0.1M, pH 7.4) hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer after 10 min of incubation at 37ºC against a blank. The percentage of inhibition of hydrogen peroxide was calculated using the following formula.

% inhibition H_2O_2= ([A0]-[A1])/ [A0] X 100

Where (A0 – Absorbance of control; A1 – Absorbance of sample)

Total antioxidant capacity: The total antioxidant capacity of the crude extracts of plant materials was evaluated by the method of Prieto *et al*. (1999). The antioxidant capacity of the extracts was measured spectrophotometrically using phosphomolybdenum method, based on the reduction of Mo (VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate / Mo(V) compounds. A 0.3 ml aliquot of sample solution was combined with 2.7 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium

phosphate and 4 mM ammonium molybdate). All samples were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank.

Reducing power assay: The reducing power of the extracts was determined according to the method of Oyaizu (1986). Plant extracts and standard antioxidants in 1 ml of distilled water were mixed separately with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL; 10 g/l). The mixtures were incubated at 50°C for 20 min. Then, a portion of TCA (10%; 2.5 ml) was added to each mixture and centrifuged at 3000 rpm for 20 min. Finally, the supernatants (2.5 ml) were mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml; 0.1%). The absorbance of the solutions was measured at 700 nm. A higher absorbance of the reaction mixture indicated that the reducing power had increased.

Results and Discussion :

The phytochemical screening and antioxidant activity of the methanolic and aqueous extracts of *Vinca rosea* and *Thevitia peruviana* revealed the presence of phenols, flavanoids , tannins, alkaloids, terpenoids, steroids, catechins and cardiac glycosides..

Phytochemical screening: Phytochemical screening revealed the presence of steroids, flavonoids, alkaloids, terpenoids, saponin, catechin, cardiac glycosides and tannins in plants selected (Table1).These phytochemicals ultimately contribute to the antioxidant property of the plants. Depending upon the concentration of these phytochemicals, the antioxidant property of plants varies.

Table 1. Phytochemical Screening of plant material

Phytochemiclas	Vinca rosea				Thevitia peruviana			
	Leaf		Flower		Leaf		Flower	
	Aq	Methanol	Aq	Methanol	Aq	Methanol	Aq	Methanol
Alkaloids	–	+	–	+	–	+	–	+
Flavanoids	+	+	+	+	–	–	–	–
Tannins	+	+	–	–	+	+	+	+
Saponin	–	+	–	+	–	–	–	–
Catechins	–	–	+	–	–	–	–	–
Terpenoid	–	+	–	+	–	+	–	+
Steroid	–	+	–	–	–	–	–	–
Cardiac glycosides	–	+	–	–	–	+	–	–

+: indicates presence, – :indicates absence, Aq = Aqueous

In *Vinca rosea* leaf all phytochemicals were present except catechins in methanolic extract but, in aqueous extract all were absent except flavanoids and tannins. In flower of *vinca rosea* alkaloids, flavanoids, saponin, terpenoid were present and rest were absent in methanolic extract but, in aqueous extract only flavanoids and catechins were present. In *Thevitia peruviana* leaf flavanoids, saponin, catechins, steroid were absent in methanolic extract but, in aqueous extract only tannins were present. However, in *Thevitia peruviana* flower alkaloids, tannins and terpenoids were present in methanolic extract but in aqueous extract again only tannins were present.

Hydrogen peroxide scavenging activity of plant extracts: Of the two, *Vinca rosea* showed less H_2O_2 % inhibition as compared to *Thevitia peruviana* . Methanolic and aqueous extract of *Thevitia peruviana* leaf showed 70.97 %and 43.24%.whereas those of *Vinca rosea* leaf showed 68.12% and 55.42% inhibitions, respectively. *Vinca rosea* flower showed 49.6% and 48.9% inhibition in methanolic and aqueous extracts. Again methanolic and aqueous extracts of *Thevitia peruviana* flower showed 47.27% and 65.4% inhibition, respectively (Fig. 1 & 2).

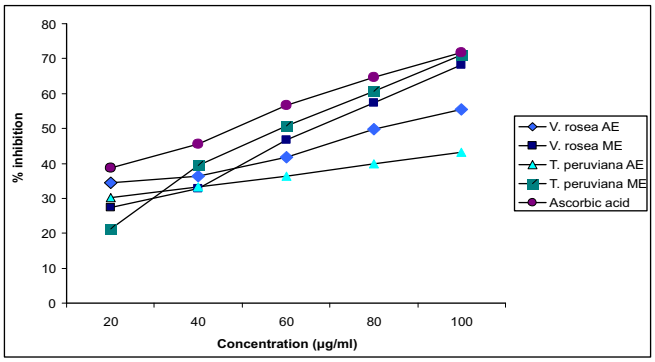

Fig 1. H_20_2 Scavenging activity of leaf extracts of plants

H_20_2Scavenging activity of methanolic leaf extracts of *Thevitia peruviana* is more than that of *Vinca rosea* whereas this activity is more in aqueous extract of *Vinca rosea* than in *Thevitia peruviana*. Both *Thevitia peruviana* and *Vinca rosea* showed less activity as compared to ascorbic acid used as standard.

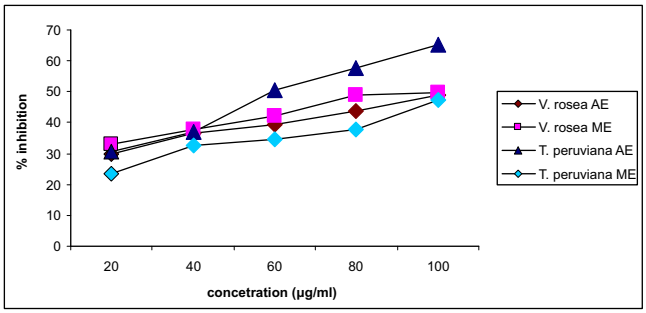

Fig 2. H_20_2 Scavenging activity of flower extracts of plants

H_20_2 Scavenging activity of aqueous extract of *Thevitia peruviana* flower was found to be more than *Vinca rosea* whereas this activity was more in methanolic extract of *Vinca rosea* than in *Thevitia peruviana*.

The measurement of H_20_2 scavenging activity is one of the useful methods of determining the ability of antioxidants to decrease the level of pro-oxidants such as, H_20_2. It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells. The inhibitive effect of plant extracts were subjected to hydrogen peroxide scavenging assay and were found to be moderate when compared to ascorbic acid taken as standard.

Total antioxidant capacity:

Table 2. Total antioxidant capacity of leaf extracts of plants as compared to the standard

Plants	V. rosea (Ascorbic Acid Equivalent)		T. peruviana (Ascorbic Acid Equivalent)	
Concentrations (mg/ml)	Aqueous Extract	Methanolic Extract	Aqueous Extract	Methanolic Extract
20	0.66	1.66	0.69	1.05
30	0.69	2.29	0.71	2.28
60	0.70	3.11	0.94	3.29
80	0.75	5.16	1.05	3.33
100	0.99	7.43	1.47	4.97

Total antioxidant capacity of methanolic leaf extract of Vinca *rosea* was more than that of *Thevitia peruviana* whereas aqueous extract of *Thevitia peruviana* has more Total antioxidant capacity than *Vinca rosea* (Table 2).

Table 3. Total antioxidant capacity of flower extracts plants as compared to the standard

Plants	V. rosea (Ascorbic Acid Equivalent)		T. peruviana (Ascorbic Acid Equivalent)	
Concentrations (mg/ml)	Aqueous Extract	Methanolic Extract	Aqueous Extract	Methanolic Extract
20	0.62	0.82	0.76	1.65
30	0.68	0.83	0.90	1.92
60	0.71	0.91	1.09	2.03
80	0.82	1.10	1.29	2.52
100	0.97	1.27	1.47	2.95

Total antioxidant capacity in aqueous and methanolic extract of *Thevitia peruviana* flowers were more to that of *Vinca rosea.* Therefore, *Thevitia peruviana* has more Total antioxidant capacity than *Vinca rosea.* (Table 3)

The results presented indicate that the antioxidant activity of plant extracts seems to be due to the presence of polyphenols, flavonoid and

anthocyanoside that may act by donating electrons and free radicals. The antioxidant capacity of a compound depends on its ability to donate hydrogen and /or electrons. Compounds like ascorbic acid, phenolics , aromatic amines, etc act as good antioxidant due to their ability to donate hydrogen/ electrons. (Prieto *et al*., 1999)

Reducing power assay:

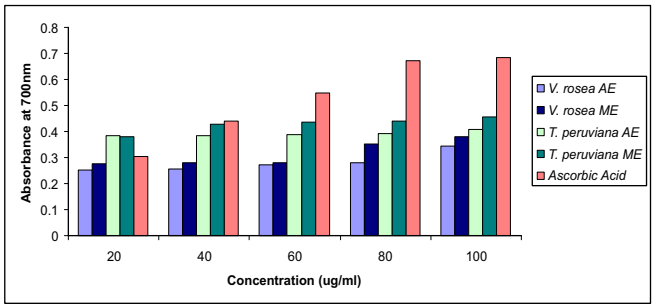

Fig 3. Reducing power assay of leaf extract of plants

Reducing power assay of both aqueous and methanolic leaf extract of *Thevitia peruviana* has been found to be more than that of *Vinca rosea* as compared to the standard. (Fig.3). In case of flower, both aqueous and methanlic extracts of Vinca rosea showed more reducing activity than that of Thevitia peruviana (Fig. 4).

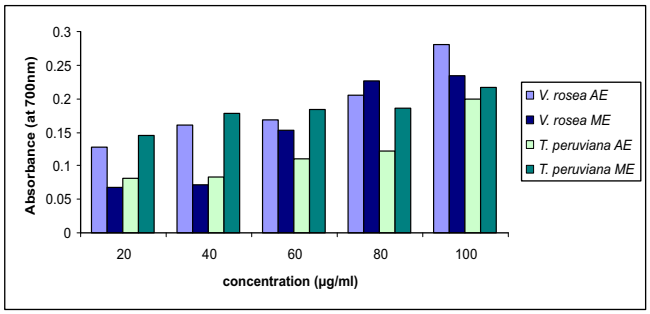

Fig 4. Reducing power assay of flower extract of plants

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Reducing potential is generally associated with the presence of reductants such as, antioxidant substances, which cause a reduction in the Fe^{3+}/ferricyanide complex to Fe^{2+}.

The reducing power capacities of the samples were compared to ascorbic acid. All the plant extract showed more or less significant reducing power at the same level as standard antioxidant. Extracts containing the highest amounts of total phenolics had weaker reducing power than compounds although results were close. Similar relations between Fe^{3+} reducing activity and total phenol content have been reported.

Conclusion :

The results of the present study suggest that the Antioxidant property of *Thevitia peruviana* is nearly equal to *Vinca rosea* which is a well known medicinal plant. Hence this member of the family can also be considered for antioxidant source. It is suggested to identify exact phytochemical responsible for its antioxidant activity.

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